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DOI: <https://doi.org/10.17236/sat00098>

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ZORA URL: <https://doi.org/10.5167/uzh-129585>

Journal Article

Published Version

Originally published at:

Braun, Ueli; Mitchell, Katharyn J; Schramm, S; Nogler, S; Hatt, Jean-Michel; Malbon, A (2016). Ultrasonographic findings in two sheep with enzootic calcinosis. *Schweizer Archiv für Tierheilkunde*, 158(12):827-832.

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Ultrasonographic findings in two sheep with enzootic calcinosis

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Summary

This report describes 2 sheep with enzootic calcinosis characterized by abnormal cardiovascular and respiratory findings and ascites causing abdominal distension. Both sheep were anorexic and listless and had increased heart and respiratory rates. Auscultation of the heart revealed a gallop rhythm in sheep 1 and a loud systolic heart murmur in sheep 2. The activities of liver enzymes were severely increased in both sheep. Abdominal ultrasonography showed severe ascites and congestion of the liver and caudal vena cava. Echocardiography in sheep 2 showed hyperechoic and markedly thickened mitral and aortic valves with moderate-severe mitral insufficiency and generalized cardiomegaly. Both sheep were euthanized and examined postmortem. In addition to ascites and pleural effusion, the principal lesions were nodular thickening of the heart valves and calcification of the aorta and other arteries. Nutrition of the sheep did not include hay pellets, but the sheep were kept together with alpacas and llamas and had access to the hay pellets of these animals. In addition visitors were allowed to feed the sheep with hay pellets offered by the zoo in a dispenser. The two types of hay pellets had Vit D concentrations of 9'900 IU VitD₃/kg and 7'000 IU Vit D₂/kg, respectively. The definitive diagnosis was enzootic calcinosis.

Keywords: sheep, enzootic calcinosis, ascites, calcification, heart valves, aorta

Ultraschallbefunde bei zwei Schafen mit enzootischer Kalzinose

In der vorliegenden Arbeit werden die Befunde bei 2 Schafen mit enzootischer Kalzinose beschrieben, bei denen neben abnormen Befunden am Herz-/Kreislauf und am Atemapparat eine Umfangsvermehrung des Abdomens infolge Aszites im Vordergrund stand. Bei beiden Schafen war das Abdomen dilatiert und das Allgemeinbefinden war deutlich gestört. Die Herz- und die Atemfrequenz waren erhöht. Beim Schaf 1 war bei der Herzauskultation zudem ein Galopprrhythmus, beim Schaf 2 ein lautes systolisches Herzgeräusch zu hören. Beide Schafe wiesen stark erhöhte Aktivitäten der Leberenzyme auf. Bei der Ultraschalluntersuchung wurde ein hochgradiger Aszites festgestellt. Die Leber und die hintere Hohlvene waren gestaut. Die beim Schaf 2 zusätzlich durchgeführte echokardiographische Untersuchung ergab hyperechogene, deutlich verdickte Mitral- und Aortenklappen. Beide Schafe wurden euthanasiert und sezziert. Bei der Sektion wurden als wichtigste Befunde neben Pleuraerguss und Aszites knotig verdickte Herzklappen und Kalzifizierungen in der Aorta und weiteren arteriellen Gefässen festgestellt. Die Schafe wurden mit Alpakas und Lamas zusammengehalten und hatten Zugang zu deren Heupellets, welche 9'900 IU VitD₃/kg enthielten. Zudem wurden sie von Zoobesuchern mit in einem Dispenser zur Verfügung gestellten Heupellets gefüttert. Diese enthielten 7'000 IU Vit D₂/kg. Aufgrund sämtlicher Befunde wurde die Diagnose enzootische Kalzinose gestellt.

Schlüsselwörter: Schaf, enzootische Kalzinose, Aszites, Verkalkungen, Herzklappen, Aorta

DOI 10.17236/sat00098

Received: 19.01.2016
Accepted: 12.06.2016

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Introduction

Enzootic calcinosis is a disease complex that has been described in detail in cattle (Dirksen et al., 1970; Dirksen et al., 1971; Köhler et al., 1974; Köhler et al., 1978; Libiseller et al., 1986; Braun et al., 2000a), goats (Wanner et al., 1986; Braun et al., 2000b) and sheep (De Barros et al., 1970; Tustin et al., 1973; Gill et al., 1976; Dirksen et al., 2003; Gufler et al., 2005; García y Santos et al., 2012). The disease may be caused by plant poisoning or mineral imbalances in the soil. Excessive intake of golden oat grass (*Trisetum flavescens*) causes a metabolic disturbance that resembles hypervitaminosis D and is characterized by calcification of soft tissues, particularly the cardiovascular system (Rambeck and Zucker, 1985). In addition to large amounts of vitamin D₃, golden oat grass contains a 1,25-dihydroxy-cholecalciferol-like glycoside that mimics the calcinogenic action of vitamin D₃ (Rambeck and Zucker, 1985). Ingestion of this substance leads to uncontrolled calcium absorption from the intestinal tract resulting in soft tissue and vascular calcification. Enzootic calcinosis caused by golden oat grass is most prevalent in alpine foothill regions where environmental conditions favour the growth of this plant over others. The most evident clinical signs include weight loss, moderate to severe locomotor disturbances and a marked drop in milk production. Affected animals have shifting limb lameness and often are unable to rise and remain on their knees. Tachycardia and systolic heart murmurs have been described in affected goats (Braun et al., 2000b). Cardiovascular and respiratory findings are common findings in sheep, in which dyspnoea alone (Gill et al., 1976) or combined with cardiovascular problems (Dirksen et al., 2003; Gufler et al., 2005) or peracute heart failure (Dirk-

sen et al., 2003) have been reported. Abdominal distension caused by ascites has not been reported in sheep with enzootic calcinosis and therefore, the goal of this case report was to describe the clinical and ultrasonographic findings in 2 sheep with enzootic calcinosis. The 2 sheep were part of a 4-head flock housed at a local zoo. The sheep were kept together with alpacas and llamas. Their own diet did not include hay pellets, but the sheep had access to the hay pellets of the new world camelids. In addition visitors were allowed to feed the sheep with hay pellets offered by the zoo in a dispenser. The two types of hay pellets had Vit D concentrations of 9'900 IU VitD₃/kg and 7'000 IU Vit D₂/kg, respectively. The sheep were referred to our clinic two weeks apart by the attending veterinarian.

Case presentation

Sheep 1 was a 4.2-year-old Ouessant ewe in advanced pregnancy. The ewe had separated from the flock several days before referral, was often recumbent and had a rapid respiratory rate (Fig. 1). Sheep 2 was a 5-year-old ram of the same breed with a similar history (Fig. 2). On admission to the clinic, both sheep were anorexic and listless and had abdominal distension. The heart rate was markedly increased at 92 beats per minute (bpm) with a gallop rhythm in sheep 1 and 196 bpm in sheep 2. Auscultation of the heart in sheep 2 revealed a grade 5/6 systolic heart murmur, which was loudest at the left heart base. There was a marked increase in the respiratory rate (80 breaths per minute) and increased breath sounds in both sheep. Rumen motility was severely reduced or absent in both sheep, and there were no faeces in the rectum of sheep 1. Based on these findings, a



Figure 1: Four-year-old Ouessant ewe (sheep 1) with abdominal distension attributable to ascites.



Figure 2: Five-year-old Ouessant ram (sheep 2) with abdominal distension attributable to ascites.

tentative diagnosis of a pulmonary disorder was made in sheep 1 and of a cardiac disorder in sheep 2.

Both sheep had leukopenia, hypoproteinaemia and hypofibrinogenaemia and severely increased activities of aspartate aminotransferase (ASAT), glutamate dehydrogenase (GLDH), sorbit dehydrogenase (SDH) and γ -glutamyl transferase (γ -GT) (Tab. 1). Sheep 1 also had hypokalaemia, hypocalcaemia and increased activity of the muscle enzyme creatine kinase (CK), sheep 2 had mild haemoconcentration.

The ultrasonographic examination of the lung and of the abdomen showed pleural effusion in sheep 2 and severe ascites in both sheep (Fig. 3). There was hepatic congestion and blunting of the angle between the diaphragmatic and visceral surfaces of the liver. The caudal vena cava was dilated and thus had a round rather than a triangular shape in cross-section (Braun and Hausmann, 1992) (Fig. 4). The portal vein was circular in cross-section with normal stellate ramifications into the liver parenchyma. The 3 layers of the wall of the gallbladder (mucosa, muscularis and serosa) were distinct in both sheep, most likely because of oedema (Fig. 5). Echocardiography, only performed in sheep 2, revealed markedly thickened, hyperechoic mitral and aortic valves (Fig. 6). There was generalized cardiomegaly and moderate to severe mitral insufficiency. The ECG obtained during echocardiography showed occasional ventricular premature complexes in addition to sinus tachycardia.

Both sheep were euthanized because of a grave prognosis and examined postmortem. Post mortem examination

Table 1: Haematological and biochemical results in 2 sheep with enzootic calcinosis.

Variable	Sheep 1	Sheep 2	5–95%-quantile ¹
Haematocrit (%)	36	39	27–38
Total leukocyte count ($\times 10^3/\mu\text{l}$)	3.8	3.4	4.5–11.4
Total protein (g/l)	48	58	62–75
Fibrinogen (g/l)	1	1	1–7
Urea (mmol/l)	15.3	5.4	2–7
ASAT (U/l)	1031	177	72–126
GLDH (U/l)	102	110	6–76
SDH (U/l)	90	122	15–57
γ -GT (U/l)	192	305	19–63
CK (U/l)	876	73	86–208
Sodium (mmol/l)	151	153	149–159
Potassium (mmol/l)	3.6	5.0	4.6–6.5
Chloride (mmol/l)	108	112	104–117
Calcium (mmol/l)	2.09	2.46	2.3–2.8
Inorg. phosphorus (mmol/l)	2.12	1.33	1.2–2.3
Magnesium (mmol/l)	1.02	0.88	0.8–1.1

¹ Tschuor et al. (2008)

was very similar in both sheep: Both had mild pleural effusion and severe ascites. The left atrioventricular and aortic valves were thickened and had a granular to nodular appearance, and in sheep 1, the aortic intima was covered with granular white plaques that extended from the heart to the iliac arteries (Fig. 7). In sheep 2, the aortic valve, the left atrioventricular valve and the chordae tendineae had hard white calcifications with a diameter of 2 to 4 mm, and multifocal to confluent, rough indurated areas were observed along much of the aorta, of greater severity within the abdominal portion (Fig. 8).



Figure 3: Abdominal ultrasonogram of sheep 1 in advanced pregnancy showing the gravid uterus and the liver surrounded by ascites fluid. 1 Ventral abdominal wall, 2 Ascites, 3 Liver, 4 Uterus, Cr Cranial, Cd Caudal.

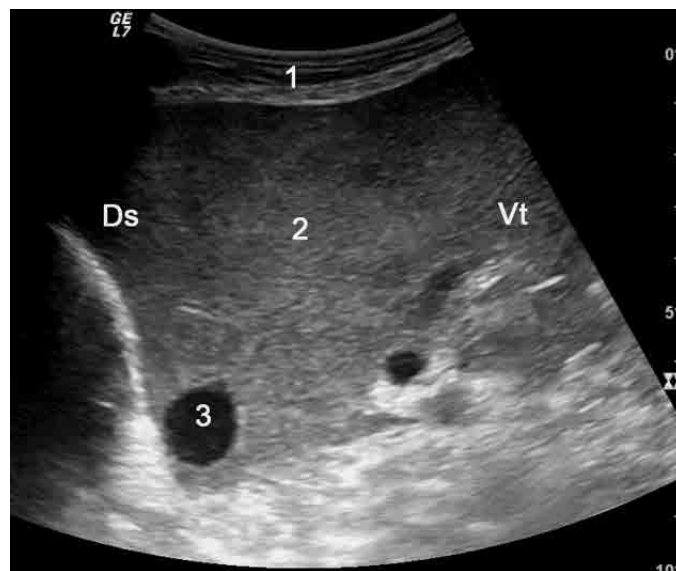


Figure 4: Ultrasonogram of the liver of sheep 1 showing the dilated circular caudal vena cava. Liver congestion is due to right-sided heart failure. 1 Abdominal wall, 2 Liver, 3 Caudal vena cava, Ds Dorsal, Vt Ventral.

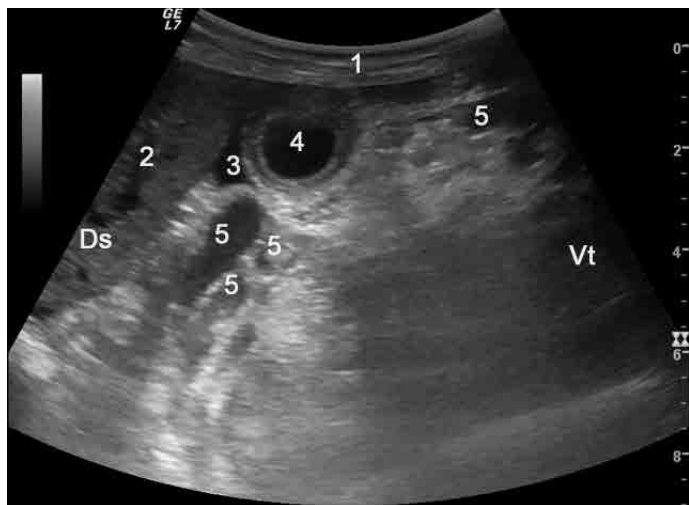


Figure 5: Abdominal ultrasonogram of sheep 2 showing an oedematous gallbladder wall attributable to right-sided heart failure. 1 Abdominal wall, 2 Liver, 3 Ascites, 4 Gallbladder with marked thickening of the wall, 5 Small intestines in cross-section, Ds Dorsal, Vt Ventral.

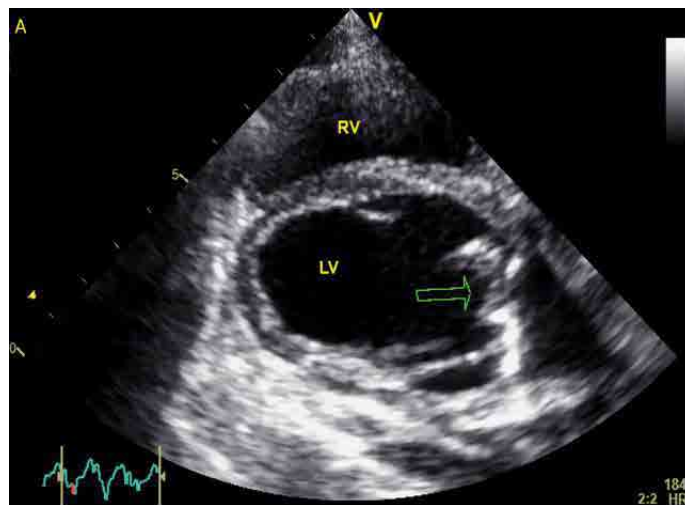


Figure 6 A: A two dimensional B-mode image, right parasternal long axis view focused on the ventricles showing the hyperechoic and thickened mitral valve (arrow) of sheep 2. RV Right ventricle, LV Left ventricle, AO Aorta, PA Pulmonary artery, LA Left atrium, RA Right atrium, V Marker for the probe – indicating that the right side of the image is dorsal.

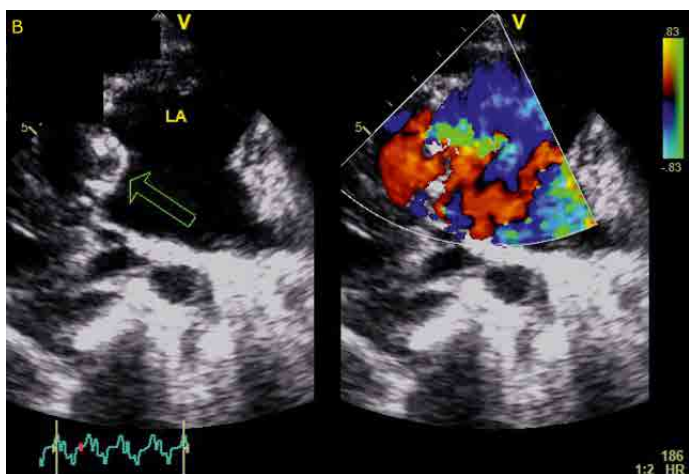


Figure 6 B: A two dimensional B-mode image (left panel) with simultaneous colour Doppler (right panel) left parasternal long axis view of the LA of sheep 2. Colour flow mapping indicates turbulent flow (colour coded in green) consistent with mitral regurgitation. The thickened, hyperechoic mitral valve is indicated (arrow). For key see Figure 6 A.

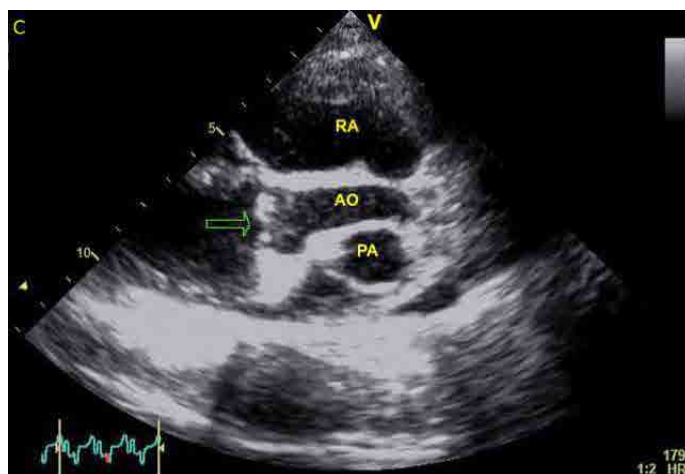


Figure 6 C: A two dimensional B-mode image in the right parasternal long axis, left ventricular outflow track view of sheep 2. The thickened and hyperechoic aortic valve is indicated (arrow). For key see Figure 6 A.

The trachea had small white inclusions in the mucosa adjacent to the tracheal rings. There were disseminated white indurations in the lungs, most prominently in the apical lobes. Histology of grossly affected areas demonstrated multifocal basophilic deposits of granular, acellular material (mineralisation) with progression to bone formation (osseous metaplasia) in the worst affected regions (Fig. 9). The mineralisation affected both the tunica intima and media of multiple arteries including the aorta, iliac and coronary arteries. There was mild sclerosis of the coronary arteries and osseous metaplasia in the lungs. Sheep 1 exhibited centrilobular degeneration and necrosis and sheep 2 severe hepatic congestion. Sheep 2 also had mild mineralisation and cartilagenous

metaplasia within the flexor tendons of the forelimbs. The final diagnosis was calcification of the aorta, arteries and heart valves attributable to enzootic calcinosis, presumably as a result of excessive ingestion of golden oat grass (*Trisetum flavescens*). Feed analysis showed that the hay pellets had a vitamin D content of 8'000 IU/kg. A botanical analysis of the hay fed to the sheep revealed a concentration of *Trisetum flavescens* below 20%. This was corroborated by chemical analyses that showed Vit D concentrations below 100 IU/kg.

Discussion

The cardinal clinical signs in both sheep were tachypnoea and tachycardia. Laboratory testing revealed increased liver enzyme activity and ultrasonography showed ascites. In sheep 2 there was evidence of congestive heart failure presumed secondary to the valvular mineralisation and fibrosis and resultant chronic bilateral valvular insufficiency and secondary hepatic congestion. Endocarditis was a plausible tentative diagnosis in sheep 2, but in sheep 1 the aetiology of the disorder was obscure. Enzootic calcinosis was not suspected in either sheep because typical locomotor signs described by several authors (Tustin et al., 1973; Dirksen et al., 2003; Gufler et al., 2005) were absent. However, cardiovascular signs are much more characteristic of enzootic calcinosis in sheep than in cattle and goats (Tustin et al., 1973; Dirksen et al., 2003; Gufler et al., 2005). Progressive circulatory and respiratory impairment, leading to heart murmurs and dyspnoea, and musculoskeletal abnormalities including mild carpal flexion, abnormally straight hind limbs, shifting of weight from foot to foot and walking or grazing on the knees are characteristic of ovine enzootic calcinosis (Dirksen et al., 2003; Gufler et al., 2005). Of 12 sheep with this condition, 4 died from acute heart failure and 6 were euthanized because of chronic circulatory insufficiency ($n = 5$) or severe lameness ($n = 1$) (Dirksen et al., 2003). Other authors observed dyspnoea (Gill et al., 1976), abnormal heart rates and auscultatory findings (Gufler et al., 2005) and heart failure (Tustin et al., 1973) in affected sheep. Clinical evidence of cardiovascular disease has also been documented in goats (Braun et al., 2000b); 3 of 6 goats with enzootic calcinosis had tachycardia with heart rates between 120 and 140 bpm. Two of these goats had a loud holosystolic heart murmur and the other had a cardiac arrhythmia.

The principal laboratory finding was a severely increase in liver enzyme activity, which was interpreted as a result of liver congestion caused by right-sided heart failure. Of note, hyperphosphataemia and high-normal calcium concentrations, which are considered characteristic of calcinosis and were described in affected sheep (Dirksen et al., 2003), were not seen in our 2 cases. In another report (Gufler et al., 2005), 3 of 5 sheep with enzootic calcinosis had hypercalcaemia but only one of these had hyperphosphataemia.

The most prominent ultrasonographic findings in 5 Merino sheep with enzootic calcinosis were semilunar valve stenosis and calcification of the carotid arteries, kidneys and lungs (Gufler et al., 2005). One of the sheep also had ascites. Based on the ultrasonographic findings of the 2 sheep described in the present report, enzootic calcinosis was not considered in the differential diagno-



Figure 7: Calcification of the aorta evidenced by numerous plaques lining the aortic intima of sheep 1 with enzootic calcinosis

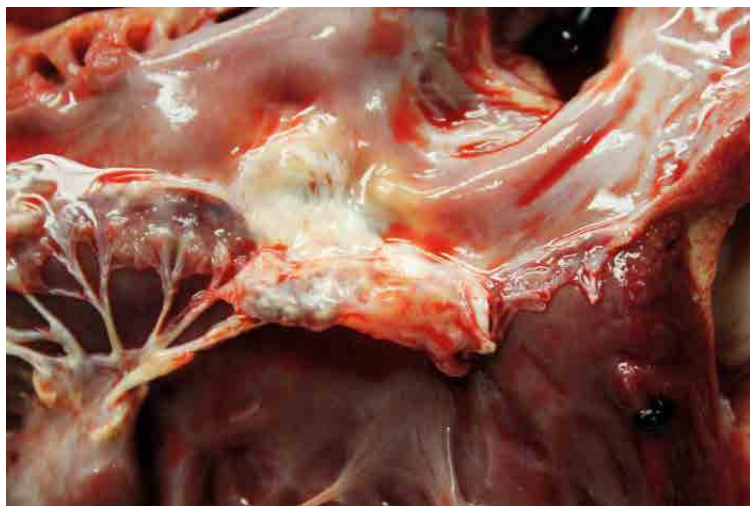


Figure 8: Calcification of the left atrioventricular valve in sheep 2 with enzootic calcinosis.

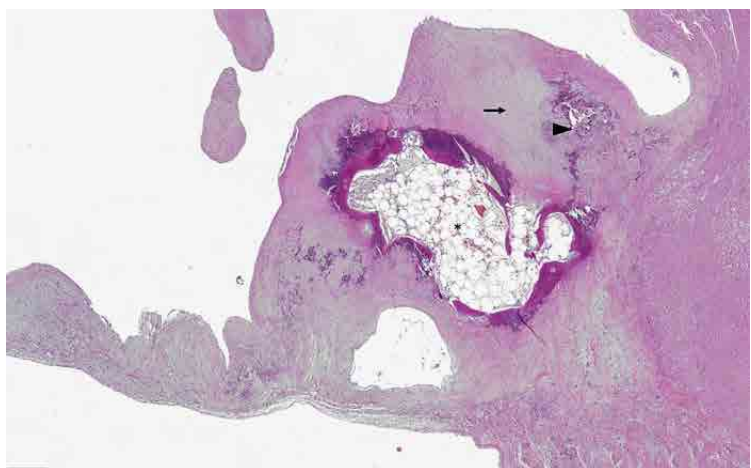


Figure 9: Attachment of the left atrioventricular valve of sheep 2 showing mineralisation (arrowhead), cartilaginous metaplasia (arrow) and advanced osseous metaplasia with bone marrow formation (*). HE x4.

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sis. In sheep 2, echocardiography showed thickening of the mitral and aortic valves, which was erroneously interpreted as endocarditis. Based on current knowledge of the typical pathological changes affecting the heart valves of sheep with calcinosis, this disease should be considered part of the differential diagnosis in sheep with ultrasonographic evidence of thickening of the heart valves. The ascites, the circular cross-section of the caudal vena cava and blunting of the liver angle were indicative of liver congestion due to right-sided heart failure. Based on findings in cattle, the differential diagnosis should also include thrombosis or compression of the caudal vena cava in sheep with these ultrasonographic findings (Braun, 2008). The severe calcification of the aorta and various heart valves in both sheep and the calcification of the trachea, lungs and flexor tendons of the forelimbs in sheep 2 largely agree with findings reported by others (Gill et al., 1976; Dirksen et al., 2003; Gufler et al., 2005).

Conclusion

In sheep with an increased incidence of cardiovascular problems accompanied by dyspnoea, enzootic calcinosis should be included in the differential diagnosis. Echocardiography is a suitable tool for identifying calcification of heart valves. In a flock with a suspected outbreak of enzootic calcinosis, a diseased sheep should be submitted for postmortem examination to confirm cardiovascular calcification. If an individual animal is affected, a small piece of a branch of the auricular artery may be removed, as proposed by Gufler et al. (2005), and examined histologically for calcification of its wall.

Acknowledgements

The authors thank Prof. Marcus Clauss, Dr. Fabia Wyss and Professor Annette Liesegang for their technical support and expertise.

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